Pestic. Sci. 1997, **50**, 291–296

A New Group of Plant-Derived Naphthoquinone Pesticides*

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(Received 5 November 1996; revised version received 22 April 1997; accepted 2 May 1997)

Abstract: A series of compounds with structures based on insecticidal/acaricidal naphthoquinones isolated from *Calceolaria andina* has been synthesised. A feature of the series is the lack of resistance shown by strains resistant to established classes of pesticides. The importance for activity of the tetra-substituted carbon atom in the side-chain, as observed in the natural products, has been investigated. In analogues with acyclic side-chains the position of the tetra-substituted carbon for optimum activity was dependent on the length of the side-chain. With cyclic side-chains, activity was dependent on the size of the ring, the number and position of the substituents therein. Activity of the compounds examined was particularly high against *Bemisia tabaci* and *Tetranychus urticae* in direct-contact tests, but was much lower than expected in leaf-dip tests. A partial improvement based on formulation has been demonstrated.

Pestic Sci., **50**, 291–296, 1997
No. of Figures: 3. No. of Tables: 7. No. of Refs: 15

Key words: naphthoquinones, insecticides, acaricides, structure–activity relationships, natural products

1 INTRODUCTION

From a collaborative programme involving the University of Chile, the Agrochemical Evaluation Unit at the University of Southampton and the Jodrell Laboratory at Kew, the insecticidal/acaricidal and fungicidal activity in extracts of *Calceolaria andina* was recognised and shown to be due to the presence in the processed extract of two naphthoquinones[1] (**1** and **2**, Fig. 1). Activities of both compounds were of a similar order to those of some established insecticides against the sucking pests *Bemisia tabaci* Genn (whitefly) and *Tetranychus urticae* Koch (two-spotted spider mite) (Table 1). Both compounds exhibited a particularly favourable property with regard to resistant insects as exemplified in Table 2. Strains of *B. tabaci* and *T. urticae* that are strongly resistant to established chemical classes (e.g. pyrethroids and organophosphates)[2,3] showed negligible resistance to the new compounds.

TABLE 1
Relative Activities of Known Compounds against Whiteflies (*Bemisia tabaci*) and Mites (*Tetranychus urticae*)

Compound	*Relative activities*	
	B. tabaci	T. urticae
1	100[a]	100[b]
2	150	180
3	7	13
4	7	80
5	39	110
Bifenthrin	1400	170
Cypermethrin	44	—
Dicofol	—	240

[a] LC_{50} 7 mg litre^{-1}, glass vial.
[b] LC_{50} 51 mg litre^{-1}, micro-immersion.

* Based on a paper presented at the meeting 'Advances in the Chemistry of Crop Protection', organised by P. J. Crowley, G. Mitchell, G. Keen, J. Pickett and P. D. Riordan on behalf of the SCI Pesticides Group and the RSC Biological & Medicinal Chemistry Group and held on 9–11 September 1996 at Churchill College, Cambridge.
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Contract grant sponsor: British Technology Group; Biotechnology and Biological Sciences Research Council.

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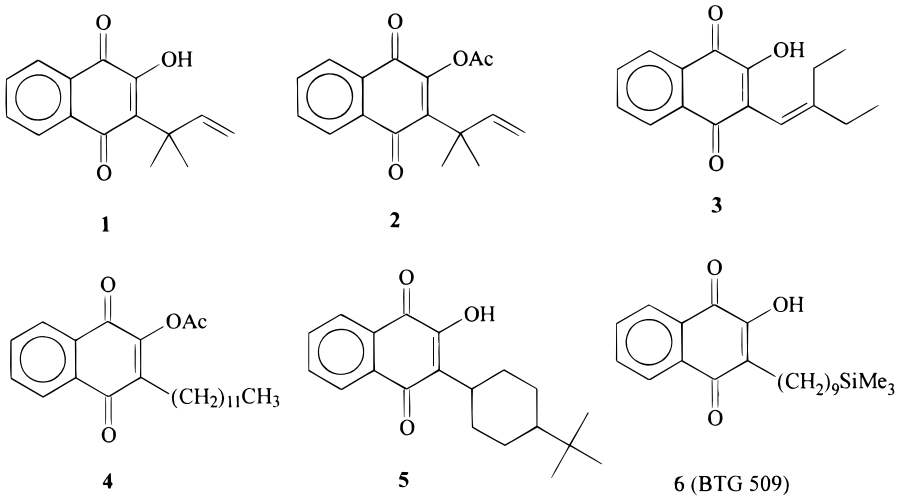


Fig. 1. Structures of compounds discussed.

Previous publications on the pesticidal activity of naphthoquinones cover compound **3**,[4] compound **4**[5] and compound **5**.[6] In bioassay procedures reported here, these compounds were generally less active than the natural products (see Table 1). A distinctive feature in the structure of the two natural products (**1** & **2**) is the quaternary (tetra-substituted) carbon at C-1 in the side-chain at position 3 of the parent naphthoquinone. The aim of the present study was to investigate the importance of this feature on the spectrum and levels of arthropodicidal activity by removing it from the natural product (**1**) and by introducing it into the three types of previously reported compounds (**3**, **4** & **5**).

TABLE 2
Resistance Factors[a] of Natural Naphthoquinones and Other Pesticides

Compound	T. urticae	B. tabaci
Cypermethrin	—	400
Bifenthrin[b]	190	—
Chlorpyrifos[b]	360	—
Profenofos[c]	—	63
1[b]	0·9	1·9
2[b]	1·6	0·7

[a] As defined in Section 2.2.
[b] Direct (micro-immersion or vial) bioassays.
[c] Leaf-dip bioassays.

2 EXPERIMENTAL

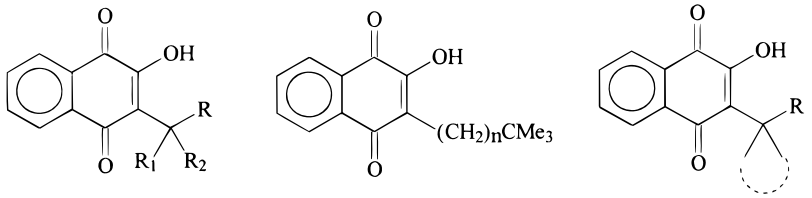
2.1 Synthesis

The compounds synthesised comprised three series, corresponding to the general formulae shown in Fig. 2.

The synthetic routes used for these series are summarised in the schemes shown in Fig. 3. Compounds in series 1 were synthesised either by reacting 2-hydroxy-1, 4-naphthoquinone (Lawsone) with the appropriate allylic alcohol under Mitsunobu reaction conditions with subsequent Claisen rearrangement[7] (see Fig. 3, scheme 1), or by reacting acylated Lawsone with the corresponding carboxylic acid in a peroxysulfate-mediated radical decarboxylation reaction (see Fig. 3, scheme 2).[8] All the compounds in series 2 and 3 were synthesised by the latter method. In some cases, the Hooker oxidation with alkaline permanganate[9] or hydrogen peroxide[10] was used to provide homologues with one less carbon at C-1 in the side chain (Fig. 3, scheme 3).

2.2 Biological testing

The susceptible and multi-resistant strains of *T. urticae* (GSS and NYRBIF) and *B. tabaci* (SUDS and NED3) have been described previously.[3,11] The strain of *Myzus persicae* (peach-potato aphid)[12] tested (USIL) was a



Series 1 Series 2 Series 3

Fig. 2. General structures of compounds synthesised.

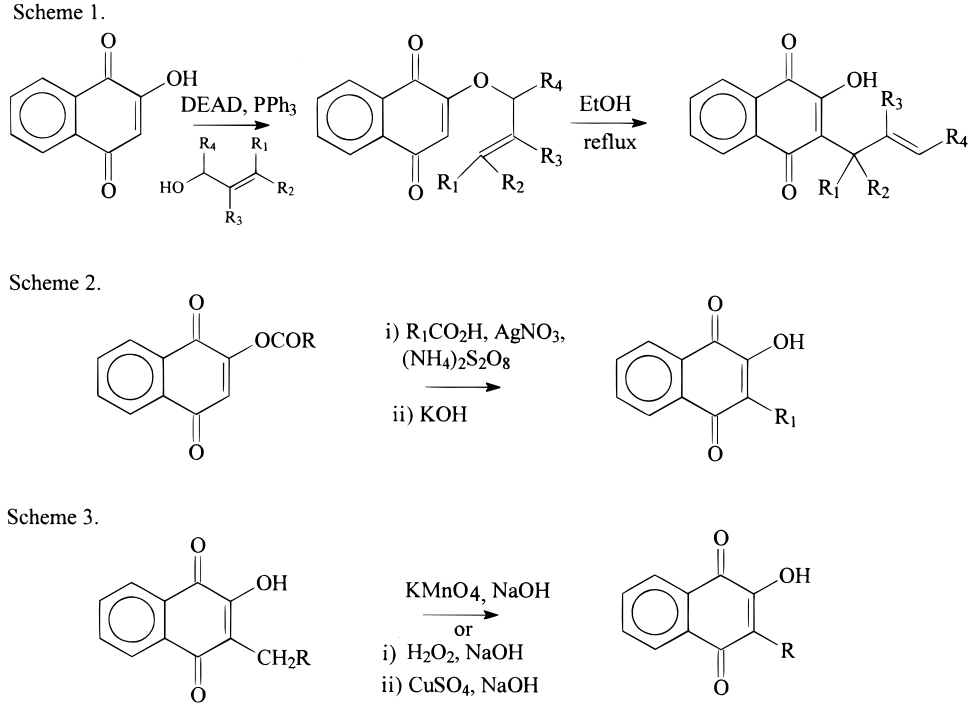


Fig. 3. Synthetic routes used.

laboratory reference clone susceptible to all aphicide classes.

Bioassay protocols used in the present study have been reported in full elsewhere. The micro-immersion assay for *T. urticae*[13] involved immersing adult mites briefly in an aqueous suspension of the test compound. In the glass-vial assay for *B. tabaci*,[14] adult whiteflies were exposed in a vial which had been precoated with the test compounds. The topical bioassay for *M. persicae* entailed applying a 0·25 μg droplet of chemical in acetone to the dorsal surface of apterous adults. In all cases, treated individuals were transferred to fresh leaf discs of either broad bean (for *T. urticae*), cotton (for *B. tabaci*) or chinese cabbage (for *M. persicae*) and mortality assessed after 72, 48 and 72 h respectively. Leaf-dip assays[2] were conducted by dipping leaf discs from the above plants into serial dilutions of formulated test material and air drying before transferring test organisms to them. Mortality was assessed after the same holding times as before.

For leaf-dip assays, the formulation (A) was prepared by dissolving the test compound (4 parts by weight) in Solvesso^{R} (Multisol Ltd, UK) (14 parts) containing the surfactants (Atlox 4851B (1 part) and Atlox 3400B (1 part)) (ICI Surfactants, UK).

All dose-response assays encompassed at least four test concentrations with a minimum of two replicates of 15 individuals per concentration. Data were subjected to probit analysis to obtain LC_{50} estimates, whose fiducial limits typically spanned a three-fold range of concentrations tested. Resistance factors are expressed as LC_{50} relative to that of the corresponding susceptible strain.

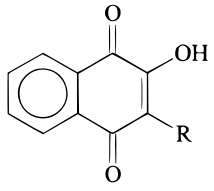
3 RESULTS AND DISCUSSION

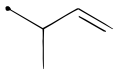
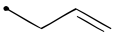
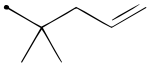
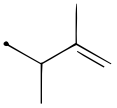
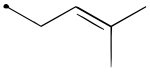
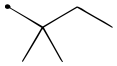
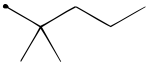
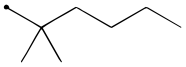
The results from the various bioassays are presented in Tables 3 to 7. Where indicated, activities are quoted as relative to compound **1** (=100).

For the compounds in series 1 (see Table 3), the importance of the gem-dimethyl group at C-1 in the side-chain of compound **1** for activity was confirmed by progressive loss of activity in compounds with only one (**7**) or no (**8**) CH_3 groups there. Insertion of a CH_2 between the ring and the gem-dimethyl group (**9**) or after the latter (**10**) also led to loss in activity. Introduction of one methyl at C-2 in **7** (to give **11**) or two methyls at C-3 of **8** (to give **12**), changes which maintained the molecular weight at that of **1**, also led to reduction of activity. Replacing the vinyl group with a $-CH_3$ group gave a compound (**13**) more active than the natural product **1** against *B. tabaci*, but further extension to $-C_2H_5$ (**14**), $-C_3H_7$ (**15**), or $-C_4H_{10}$ (**16**) led to progressive loss in activity against both species. The hydroxy analogue (**17**) of compound **4** was particularly effective against *T. urticae*, but when a gem-dimethyl group was introduced at C-1 (compound **18**) substantial loss of activity against both *T. urticae* and *B. tabaci* was incurred.

As in the case of **1**, stepwise insertion of CH_2 groups at C-1 in **13** (to give series 2, **19**–**29**) (See Table 4) also led to progressive loss of activity against *B. tabaci*. In contrast, the activity against *T. urticae* peaked sharply at $n = 9$ (compound **27**). A similar optimum (near $(CH_2)_{11}$) has been observed for the corresponding $-CH_3$ terminated series (see compound **4**) for activity against *T. urticae*.[5]

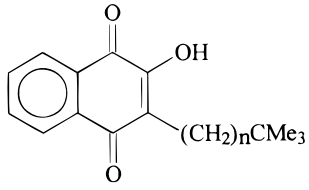
TABLE 3
Relative Activities of Compounds in Series 1



Compound number	*R*	*Relative activity*[a]	
		B. tabaci	T. urticae
7		50	61
8		39	—
9		44	77
10		NA[b]	17
11		27	10
12		42	24
13		120	62
14		110	38
15		70	55
16		67	50
17	$(CH_2)_{10}CH_3$	<10	5500
18	$(CH_2)_{10}CH_3$	NA	<10

[a] Relative activities are based on compound **1** = 100 (see Table 1).
[b] NA = not active at highest concentration tested (1000 mg litre^{-1}).

TABLE 4
Relative Activities of Compounds in Series 2



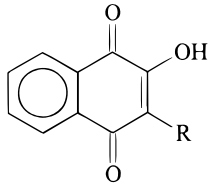
Compound number	*n*	*Relative activity*[a]	
		B. tabaci	T. urticae
13	0	120	62
19	1	64	1
20	2	100	140
21	3	44	1700
22	4	61	1500
23	5	35	520
24	6	54	100
25	7	56	1400
26	8	10	940
27	9	4	11 000
28	10	1	3600
29	11	1	500

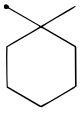
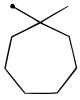
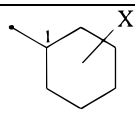
[a] Relative activities are based on compound **1** = 100.

The investigation was next extended to compounds in which the quaternary carbon in the side-chain formed part of a ring system (series 3). The results for three selected examples (**30**, **31**, **32**) (Table 5) showed that optimum activity was associated with a cyclohexyl ring system (compound **31**). Removing the methyl group at C-1 or replacing it with $-C_2H_5$, $-CF_3$ or $-CH{=}CH_2$ (compounds **33** to **36**) or changing its position of substitution in **31** (compounds **37** to **39**), generally lowered activity. Introducing an additional $-CH_3$ group in the cyclohexyl ring (compounds **40** to **42**) also appeared to reduce activity. However, these compounds were mixtures of isomers (up to 2 : 1 ratio of *syn* : *anti* isomers, except compound **37**, which was 10 : 1). Further studies are under way to isolate and test the individual diastereomers. Consistently, when a CH_3 group was introduced at C-1 to create a quaternary carbon in a Bayer type compound (**5**) to give **43**, activity also decreased substantially. Preliminary results with examples of the synthetic naphthoquinones reported here indicate that their resistance factors, assessed in the same way as for the natural compounds (Table 2) are equally low, i.e. there is no cross-resistance to established classes.

Having identified a range of highly active naphthoquinones using direct contact assays, evaluation of selected compounds was extended to leaf-dip assays (see Section 2.2) which more closely resemble field conditions. Formulation A, based on Solvesso^{R} (commonly used for lipophilic compounds, e.g. pyrethroids) and a pair of suitable surfactants, was developed (see Section 2.2). With the present compounds, emulsions stable for the duration of the bioassay were obtained. Using this formulation, compounds **1** and **2** are compared with commercially formulated samples of bifenthrin and chlorpyrifos in Table 6, using dose-transfer factors (DTFs) (see footnote to Table 6), as an empirical measure of the effectiveness of the formulation in making the compound available for pick-up by the pest. As can be seen, the DTFs for compounds **1** and **2** are

TABLE 5
Relative Activities of Compounds in Series 3



Compound number	*R*	*Relative activity*[a]	
		B. tabaci	T. urticae
30		23	104
31		7000	180
32		7	100
			
33	X=H	110	170
34	1-Et	241	NA
35	1-CF_3	29	139
36	1-CH=CH_2	780	63
37	2-Me	640	130
38	3-Me	180	50
39	4-Me	160	680
40	1-Me, 2-Me	250	170
41	1-Me, 3-Me	220	50
42	1-Me, 4-Me	35	NA
43	1-Me, 4-*t*Bu	7	NA

[a] Relative activities are based on compound **1** = 100.

close to those for the standards for *T. urticae* but are significantly lower for *B. tabaci*. Monitoring over time the vial (contact) and leaf-dip assays showed that, in the latter test, the poisoning effect was substantially slower and attenuated in comparison with the vial assay, so dose-transfer was less efficient.

TABLE 6
Performance of Naphthoquinones in Leaf-Dip Bioassays, using Formulation A, in Comparison with Direct Bioassays

Compound	B. tabaci		T. urticae	
	LC_{50}	*DTF*[a]	LC_{50}	*DTF*
1	7	14	51	130
2	4·8	17	29	75
Bifenthrin	0·3	100	86	120
Chlorpyrifos	—	—	100	71

[a] DTF = (LD_{50} (ng per insect) or LC_{50} (mg litre^{-1}) in vial or micro-immersion assay × 100)/LC_{50} in leaf-dip assay.

The problem of dose-transfer was much more pronounced with the more active synthetic analogues than with the natural products. Results for one extensively studied synthetic analogue, BTG 509 (compound **6**, Fig. 1),[15] against both the test species and *M. persicae* are shown in Table 7. As can be seen, with formulation A, DTF against *B. tabaci* is similar to that observed for the natural products (**1** and **2**) (Table 6), but in contrast DTFs against *T. urticae* and *M. persicae* are much lower. A significant increase in DTFs against the latter two species, especially *M. persicae*, was observed when another formulation (currently under patent consideration), based on a formulant of a different molecular weight, was used (Table 7). Clearly, formulation is an important consideration in the development of these compounds.

In summary, synthesis and assay of a series of pesticidal naphthoquinones has clarified the significance of a quaternary carbon atom as a structural feature in the natural compounds, and has led to the discovery of a range of synthetic compounds highly active against both susceptible and resistant strains. Their effectiveness in leaf-dip assays is lower than expected, but can be improved to some extent by using a more suitable formulation.

ACKNOWLEDGEMENTS

The authors thank Stuart Cameron and Carlos Escobar for synthesis of some of the compounds, Elizabeth

TABLE 7
Performance of Two Formulations of BTG 509 (**6**) in Leaf-Dip in Comparison with Direct Bioassays using Formulations A and B

Formulation	B. tabaci		T. urticae		M. persicae	
	LC_{50} (*ng per insect*)	*DTF*	LC_{50} (*ng per insect*)	*DTF*	LD_{50} (*ng per insect*)	*DTF*
A	20	25	0·7	0·6	29	<0·5
B		25		2·2		44

Cook, Elizabeth Simms, Mark Hedges and Fiona Guthrie for technical assistance and Norman Janes for advice and help in preparing the manuscript. This work was supported by the British Technology Group Limited. IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

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